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(54) Title: COMPOUNDS AND METHODS RELATED TO PARATHYROID HORMONE-LIKE PROTEIN

(57) Abstract

The invention provides antibodies and other epitope-specific binding molecules capable of specifically binding to an epitope or epitopes on parathyroid hormone-related protein (PTHRP) 140-173 and/or PTHRP 109-141. Other aspects of the invention involve providing various polypeptides and other molecules capable of binding to the epitope-specific binding molecules of the invention, and assays and kits for the detection of PTHRP in tissues, cells and/or fluids, and the diagnosis, monitoring and/or treatment of various diseases.

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# SPECIFICATION

## COMPOUNDS AND METHODS RELATED TO PARATHYROID HORMONE-LIKE PROTEIN

### FIELD OF THE INVENTION

The field of the invention is chemistry related to parathyroid hormone-like protein.

### BACKGROUND OF THE INVENTION

Malignancy-associated hypercalcemia, also referred to as humoral hypercalcemia of malignancy (HHM), is an important pathological sign of many forms of cancer, tumors and other neoplasms. Malignancy-associated hypercalcemia is frequently caused by the over expression of parathyroid hormone-like protein (also referred to in the literature as parathyroid hormone-related protein) (PTHRP). Parathyroid hormone-like protein stimulates bone resorption and the reabsorption of calcium in the kidneys so as to raise calcium levels in the blood to dangerous levels. Secretion of parathyroid hormone-like protein has been associated with tumors derived from numerous tissues, and PTHRP overexpression has been associated with many different types of cancer including renal, bladder, prostate, breast and ovarian cancer. A review of PTHRP can be found in Burtis, Clin. Chem. 38:2171-2183 (1992).

Human parathyroid hormone-like protein, as determined by analysis of gene structure, consists of a 173 amino acid

protein derived from a precursor molecule having a 36 amino acid leader sequence, a 1-139 amino acid protein and a 1-141 amino acid protein, as described in Suva, *et al.*, Science 237:893-896 (1987) and Magnin, *et al.*, Proc. Natl. Acad. Sci. USA 85:597-601 (1988). Unless specifically indicated otherwise, the term "PTHRP" and "parathyroid hormone-like protein" as used herein refer to human PTHRP molecules. A review of the structure of parathyroid hormone-like protein and genes encoding parathyroid hormone-like protein can be found in, among other places, Burtis, Clin. Chem. 38:2171-2183.

Parathyroid hormone-like protein undergoes several proteolytic cleavages in the body. Different regions of PTHRP can be found circulating in the bloodstream at different concentrations, Ratcliffe, *et al.*, Clin. Chem. 37:1781-1787 (1991), Burtis, *et al.*, J. Bone. Miner. Res. 6 (Suppl. 1):5229 (1991). Moreover the specific population of different PTHRP molecules may also vary in accordance with progression of the neoplasm. Thus, many different processed forms of PTHRP, i.e., "isoforms" of PTHRP, are produced in varying levels of abundance in neoplastic cells. Accordingly, antibodies specific for different regions of PTHRP produce binding signals of different strengths depending on the relative abundance of the available antibody binding site in different bodily fluids, tissues, and cells.

The variability among the different processed forms of PTHRP in an individual has important implications for PTHRP

assays, and the therapy and diagnosis of neoplasms because different antibodies specific for different regions of the PTHRP sequences including the 1-173 PTHRP sequence may vary significantly in their ability to detect the molecule. Thus, different PTHRP specific antibodies vary with respect to how well they are capable of detecting PTHRP, and consequently detecting neoplasm.

Given the frequent overexpression of parathyroid hormone-like protein in neoplastic cells, and the pathogenic effects of the production of excess levels of parathyroid hormone-like protein, it is of interest to provide antibodies for the sensitive detection of parathyroid hormone-like protein, including specific peptides of the native molecules.

## SUMMARY OF THE INVENTION

The invention provides for many different compounds and methods relating to PTHRP. One aspect of the invention provides eptitope-specific binding molecules which are capable of specifically binding to at least one epitope on PTHRP 140-173 or at least one epitope on PTHRP 109-141. The eptitope-specific binding molecules include antibodies produced by hybridomas having identifying numbers 1D5 and 9H7, respectively, and non-antibody molecules such as those prepared by molecular imprinting which have specificity similar to that of the antibodies.

Other aspects of the invention provides target molecules which bind to the eptitope-specific binding molecules. Such

target molecules include polypeptides consisting of amino acids 140-173 or 109-141 of PTHRP, or subsets thereof, and other non-peptide molecules.

Other aspects of the invention provide *in vitro* and *in vivo* assays and kits for the detection of PTHRP in neoplastic cells and in various fluids of the body, and for the diagnosis and monitoring various PTHRP-producing diseases.

Still other aspects of the invention provide methods of treating neoplasms, such as those involving administering to a patient or to the fluids or tissues of a patient, *in situ* or *ex situ*, one or more different eptitope-specific binding molecules of the invention.

## DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention exploits the surprising discovery that monoclonal antibodies raised in response to a peptide consisting of amino acid residues 140-173 of PTHRP and monoclonal antibodies raised in response to a peptide consisting of amino acid residues 109-141 of PTHRP, either alone or in combination, are able to detect PTHRP expressing neoplastic cells with a much higher sensitivity than other monoclonal antibodies that are capable of specifically binding to other regions of the PTHRP molecule, and that the detection of PTHRP with such monoclonal antibodies was better correlated with the presence of neoplasia, especially breast cancer and prostate cancer, than other PTHRP specific antibodies.

EPTITOPE-SPECIFIC BINDING MOLECULES

One aspect of the invention provides eptitope-specific binding molecules which are specific for one or more epitopes present on PTHRP 140-173. Such molecules include various antibodies, and particularly the monoclonal antibodies produced by the hybridoma 1D5 which was produced in the laboratory of Dr. Leonard Deftos, University of California, San Diego, and the San Diego Veterans Affairs Medical Center. Antibodies with binding specificities similar to the monoclonal antibody produced by hybridoma 1D5 may be obtained by, among other methods, immunizing a mouse with PTHRP 140-173 and employing conventional hybridoma generation techniques. Antibodies with binding specificities similar to the monoclonal antibody produced by hybridoma 1D5 can be confirmed by performing binding assays against PTHRP 140-173 or other assays as described below.

Another aspect of the invention provides antibodies specific for one or more epitopes present on PTHRP 109-141. Such molecules include various antibodies, and particularly the monoclonal antibodies produced by the hybridoma 9H7 which was produced in the laboratory of Dr. Leonard Deftos, University of California, San Diego, and the San Diego Veterans Affairs Medical Center. Antibodies with binding specificities similar to the monoclonal antibody produced by hybridoma 9H7 may be obtained by, among other methods, immunizing a mouse with PTHRP 109-141 and employing conventional hybridoma generation techniques. Antibodies with

binding specificities similar to the monoclonal antibody produced by hybridoma 9H7 can be confirmed by performing binding assays against PTHRP 109-141 or other assays as described below.

As used herein, the term "antibody" includes conventional antibodies such as immunoglobulin G(IgG) and M(IgM), and also refers to various antibody-derived molecules that possess binding specificity that is similar to the binding specificity of the antibody from which they were derived. Examples of such antibody-derived molecules include Fab fragments, Fabc fragments, Fab' fragments, F(ab') fragments, antibody fusion proteins, single-chain antibodies, diabodies, bifunctional antibodies, chimeric antibodies, CDR grafted humanized antibodies, and the like.

The antibodies of the invention include both monoclonal and polyclonal antibodies produced from a variety of animals including rabbits, rats, mice, monkeys and the like. Information about different types of antibody derived molecules that posses binding specificity that is similar to the binding specificity of the antibody from which the derivative is derived and methods of making them can be found in, among other places, Borreback, Antibody Engineering: 2nd Edition, Oxford Press (1994), Winter and Milstein, Nature 349:293-299 (1991), and the like. The antibodies of the invention may vary in both immunoglobulin class and allotype. Techniques for the production of conventional and monoclonal antibodies can be found among other places, Harlowe and Lane,

Antibodies: A Laboratory Manual, Cold Spring Harbor Press (1988), Peter and Baumgarten, Monoclonal Antibodies, Springer-Verlag, New York, NY (1992). Antibodies of the invention include various antibody derived molecules that may be initially isolated through *in vitro* selection techniques such as the use of combinatorial libraries that is described by Huse, *et al.*, Science 256:1275-1281 (1989) and similar techniques for *in vitro* antibody screening. Such *in vitro* selected molecules may be isolated by screening libraries with the target molecules of the invention.

As used herein, the term "eptitope-specific binding molecules" includes both antibodies as described above, and also various non-antibody molecules which have negligible structural homology to antibodies but still have the property of being able to specifically bind to PTHRP 140-173, PTHRP 104-143, or other subfragments derived from these peptides. Examples of non-antibody epitope-specific binding molecules include "molecular imprints". Descriptions of how to produce molecular imprints can be found in, many other places, taught in US Patent 5,110,833 (Mossbach), O'Shannessy *et al.*, Anal. Biochem. 177:144-149 (1989), Andersson *et al.*, J. Chromatog. 513:167-179 (1990), Andersson *et al.*, J. Chromatog. 516:313-322 (1990), Andersson *et al.*, J. Chromatog. 516:323-331 (1990), Dalulis *et al.*, Biotech. and Bioeng. 39:176-184 (1992), and Glad *et al.*, J. Chromatog. 347:11-13 (1985). Non-antibody epitope-specific binding molecules provided for herein may be substituted for the antibodies in the assays,

kits, treatment protocols and other aspects of the invention.

Another aspect of the invention provides various conjugates of the eptitope-specific binding molecules, such as those labeled with a detectable marker having a chemical or biological properties of interest. Markers of interest include radionuclides, (e.g., $^{131}I$, $^{125}I$, $^{99}Tc$), fluorophores, enzymes, chromophores, biotin, paramagnetic isotopes, colloidal gold, radio-opaque imaging compounds, and the like. Labeled antibodies of the invention find use in a variety of procedures including immunoassays and disease therapy. Techniques for labeling of antibodies, derivatives of antibodies, and other molecules are well known to the person of ordinary skill in the art and detailed descriptions and procedures for labeling can be found, among other places, in Harlowe and Lane, Antibodies: A Laboratory Manual, Coldspring Harbor Press (1988), Catty, Antibodies: A Practical Approach (Volumes 1 and 2), IRL Press, New York (1989), and the like.

Another aspect of the invention provides hybridomas for the production of monoclonal antibodies of the invention. Such hybridomas include 1D5 and 9H7 and other hybridomas producing monoclonal antibodies with similar binding properties for PTHRP epitopes. Techniques for the production of hybridomas are described, among other places, in Peter and Baumgarten, Monoclonal Antibodies, Springer-Verlag, New York, NY (1992). Conventional techniques for the production of monoclonal antibodies may be conveniently adapted for the production of hybridomas of the invention. For example, a

mouse may be inoculated with PTHRP 140-173 or other target molecules of the invention containing the appropriate epitope or epitopes so as to provide suitable fusion partners for hybridoma formation and other hybridomas producing monoclonal antibodies with similar binding properties for PTHRP epitopes.

Another aspect of the invention provides cells for the expression of the antibodies of the invention by recombinant DNA technology, i.e., cells that do not naturally produce the antibodies of the invention. The recombinant DNA expression of such antibodies may be effected through the use of polynucleotide sequences encoding such antibodies. The recombinant cells for the expression of the antibodies of the may be either prokaryotic or eukaryotic. General techniques for the recombinant production of antibodies are described in, among other places, in U.S. Patent 4,816,397 (Boss, *et al.*), U.S. Patent 4,816,567 (Cabilly, *et al.*), U.K. Patent GB 2,188,638 (Winter, *et al.*), and U.K. Patent GB 2,209,757. These general techniques for recombinant antibody production may be adapted to the antibodies of the invention by the person of ordinary skill in the art.

Another aspect of the invention provides for polynucleotide sequences encoding antibodies of the invention as well as expression vectors for the expression of the antibodies encoded by such polynucleotides. Polynucleotides of the invention encoding antibodies of the invention may be readily obtained by using recombinant DNA cloning techniques that are well known to the person of ordinary skill in the art. For

example, genes encoding antibodies specific for PTHRP 140-173 may be isolated from hybridomas capable of producing such antibodies , e.g., hybridoma 1D5, by means of nucleic acid hybridization screening of hybridoma derived libraries or by performing PCR (polymerase chain reaction). Methods for isolating genes encoding antibodies from hybridomas are described, among other places, in Orlandi, R. *et al.*, Proc. Natl. Acad. Sci. USA 86:3833-3837 (1989).

TARGET MOLECULES

Yet other aspects of the invention provide target molecules capable of specifically binding to the antibodies of the invention. Such target molecules include PTHRP 140-173, PTHRP 109-141, and subfragments and derivatives thereof. The target molecules may comprise peptide or non-peptide molecules, but are preferably peptides. A particularly preferred embodiment of a PTHRP 140-173 derived target molecule is the polypeptide PTHRP 140-173, which consists of amino acids 140-173 of PTHRP in the following sequence: Thr-Ala-Leu-Leu-Trp-Gly-Leu-Lys-Lys-Lys-Lys-Glu-Asn-Asn-Arg-Arg-Thr-His-His-Met-Gln-Leu-Met-Ile-Ser-Leu-Phe-Lys-Ser-Pro-Leu-Leu-Leu-Leu.

A particularly preferred embodiment of a PTHRP 109-141 target molecule is the polypeptide PTHRP 109-141, consisting of amino acids 109-141 of PTHRP in the following sequence: Ser-Ala-Trp-Leu-Asp-Ser-Gly-Val-Thr-Gly-Ser-Gly-Leu-Glu-Gly-Asp-His-Leu-Ser-Asp-Thr-Ser-Thr-Thr-Ser-Leu-Glu-Leu-Asp-Ser-Arg-Arg-His.

In addition to PTHRP 140-173 and PTHRP 109-141, the invention provides target molecules that are derivatives of PTHRP 140-173 and PTHRP 109-141, respectively, wherein the derivatives comprise epitopes that are able to bind to the antigen recognition sites of antibodies produced by hybridomas having numbers 1D5 and 9H7, respectively, and other antibodies of the invention. Such derivatives include peptides comprising the amino acid sequence of PTHRP 140-173 or PTHRP 109-141, and further comprising additional amino acids. PTHRP 140-173 and PTHRP 109-141 derivatives that are target molecules of the invention also include polypeptides that comprise less than the complete amino acid sequences of PTHRP 140-173 or PTHRP 109-141, respectively, and other splice variants. PTHRP 140-173 derivatives that comprise less than the complete amino acid sequence of PTHRP 140-173 may easily be obtained by systematically producing polypeptides that consist of subregions of PTHRP 140-173 and testing such subregions for the property of specifically binding to antibodies produced by hybridoma 1D5 or other antibodies of the invention. The target molecules of the invention also include PTHRP 140-173 derivatives in which one or more amino acid residue of PTHRP 140-173 is substituted by a different amino acid residue. Preferably such amino acid residue substitutions are conservative replacements, i.e, amino acid residues are substituted with amino acid residue with side chains having similar chemical properties, e.g., valine is substituted with alanine, leucine is substitute with isoleucine, etc. The

invention similarly provides for additional PTHRP 109-141 derived target molecules. The invention provides for numerous antibodies, target molecules, and assays.

It will be appreciated by the person of ordinary skill in the art that specific proteins of PTHRP 140-173 that interact with the antigen binding sites on the antibodies of the invention, particularly the monoclonal antibody may readily be determined using conventional techniques in molecular biology. For example, sequential sets of small polypeptides, e.g., about 6 amino acids, may be made by *in vitro* synthesis and test for the polypeptides that actual bind to the monoclonal antibody produced by hybridoma 1D5. The epitopes of PTHRP 104-141 that specifically interact with monoclonal antibody produced by hybridoma 9H7 may be found by analogous methods.

Target molecules of the invention may be identified by conventional immunological techniques such as antibody binding assays, e.g., competition for binding between PTHRP 140-173 and monoclonal antibodies produced by hybridoma 1D5 or other antibodies (monoclonal or polyclonal) produced in response to PTHRP 140-173. Target molecules of the invention may be isolated by a variety of screening methods well known to the person of ordinary skill in the art, wherein the screening method employs the antibodies of the invention. Such methods include the screening of random peptide libraries or libraries of peptide variants derived from PTHRP 140-173 or PTHRP 109-141. Detailed description of methods of screening libraries for peptides can be found in, among other places, in Clackson

and Wells, Trends in Biotech. 12:173-184 (1994), Kay *et al.*, Gene 128:59-65 (1993), Lane and Stephen, Curr. Opin. Immunol. 23:709-715 (1993), Keller *et al.*, Virology 193:709-716 (1993), Glaser *et al.*, J. Immunol. 149:3903-3913 (1992), and Arkin and Yonvan, Bio/Technology 10:297-300 (1992). The libraries screened for target molecules of the invention may be *in vitro* synthesized libraries or may involve the use of phage libraries. Detailed procedures for the generation and screening of peptide libraries in phage can be found, for example, in Smith, Science 228:1315-1317 (1985), Cortese *et al.*, Trends Biotech. 12:262-267 (1994), Smith, Curr. Opin. Biotech. 2:668-673 (1991), and Felici *et al.*, Gene 128:21-27 (1993).

Screening for target molecules may also be accomplished by the screening of libraries of diverse peptides with the antibodies of the invention, e.g., antibodies produced by hybridomas 9H7 and 1D5. The screening of diverse libraries of molecules with antibodies of the invention may be applied to non-peptide molecule libraries so as to isolate non-peptide molecules that specifically bind to the antibodies of the invention. The generation and screening of non-peptide libraries is described in, among other places, Devlin *et al.*, Science 249:404-406 (1990), Kay *et al.*, Gene 128:59-65 (1993), Scott *et al.*, Proc. Natl. Acad. Sci. USA 89:5398-5402 (1992), Oldenburg, Proc. Natl. Acad. Sci. USA 89:5393-5397 (1992), and Hoess *et al.*, Gene 128:43-49 (1993). Additionally, the three dimensional structure of PTHRP 140-173 and PTHRP 109-143 may

be determined (at least in part) by techniques such as nuclear magnetic resonance (NMR) and x-ray crystallography. Three-dimensional structural information of PTHRP 140-173 and PTHRP 109-141 may be used by the person of ordinary skill to design various derivatives of PTHRP 140-173 and PTHRP 109-141, respectively.

PTHRP 140-173 and PTHRP 109-141, as well as other target molecules of the invention that are peptides, may be produced *in vitro* by conventional solid phase polypeptide synthesis techniques, such as those described in the Bodanszky, Peptide Chemistry: A Practical Textbook, Springer-Verlag, New York, NY (1988), and Bailey, An Introduction To Peptide Chemistry, John Wiley & Sons, New York, NY (1992). Machines for the automated synthesis of polypeptides having desired amino acid sequences are commercially available from many suppliers, e.g., Applied Biosystems, and may be used to produce target molecules of the invention that are peptides. Additionally, PTHRP 140-173, PTHRP 109-141, and other target molecules of the invention may be produced by enzymatically or chemically cleaving PTHRP (produced naturally or by recombinant DNA technology) into smaller polypeptide fragments. Additionally, PTHRP 140-173, PTHRP 109-141, and other target molecules of the invention may also be produced by well-known recombinant DNA techniques, such as those described in Goedel, Gene Expression Technology, Methods In Enzymology Volume 185, Academic Press, San Diego (1991).

The target molecules of the invention have a number of

uses that are apparent to the person of ordinary skill in the art. For example, the target molecules of the invention may be used in immunoassays for the detection PTHRP. Target molecules of the invention may also be used produce the PTHRP-specific antibodies of the invention. Such antibodies may be produced by conventional procedures for the production of monoclonal antibodies or polyclonal antibodies, wherein an animal is injected with target molecules of the invention so as to induce the production of antibodies specific for the carboxy terminus of PTHRP.

ASSAYS AND KITS

Another aspect of the invention provides assays for the detection of PTHRP. The term "assay" as used herein is used to refer both to a method of performing an assay and the physical assay device (and/or assay reagents). The assays and assay methods provided by the invention include numerous immunoassays that have been specifically adapted for use with the antibodies and/or antibody molecules of the invention. Immunoassays may involve the use of pairs of molecules that can specifically bind to one another with high affinity and specificity. Typical of such specific binding pairs of molecules are an antibody and a molecule recognized by the binding sites on the antibody, e.g., the analyte.

Immunoassays involve the measurement of the formation of (or lack of formation) complexes between the members of a specific binding pair of molecules, e.g., an antibody and an

analyte recognized by the antibody. The immunoassay may detect analyte directly, i.e., in which complex formulation occurs between an antibody and the analyte so as to produce a signal that directly correlates with analyte concentration. Analyte concentration also may be detected by measuring the inhibition of complex formation, wherein the analyte of interferes with the formation of detectable complexes. The antibodies and target molecules of the invention may be used as the specific binding pair components in a variety of immunoassays, including but not limited to, ELISA, radioimmunoassays (RIA), EMIT® assays, IRMA, two-site and the like.

A preferred embodiment of the assays of the invention are sandwich type assays in which one of the antibodies in the sandwich is a PTHRP 140-173 specific antibody of the invention and the other antibody in the "sandwich" is a PTHRP 109-141 specific antibody of the invention. Sandwich type assays employ a first antibody to bind analyte to a solid support (including paramagnetic beads and microtitre dishes) or the like and a second antibody that has the property of binding to analyte bound by the first antibody. The amount of bound second antibody may be measured so as to provide for the quantitation of the analyte in a solution. Particular preferred antibodies for use in a sandwich assay are the antibodies produced by hybridoma 1D5 and hybridoma 9H7, when used in combination with one another. Detailed protocols for some of the many immunoassays that may readily be adapted for

use with the antibodies and/or antibody binding molecules of the invention can be found in D. Catty, Antibodies: A Practical Approach Volumes 1 and 2, IRL (1989), T. Chard, An Introduction To Radioimmunoassay and Related Techniques, Elsevier Press (1990), J. Clausen, Immunochemical Techniques For The Identification and Estimation Of Macromolecules: Laboratory Techniques In Biochemistry and Molecular Biology 3rd Ed., Elsevier Science Publishers (1988), and the like. The assays of the invention may be either quantitative or qualitative.

In another aspect of the invention, assays are performed which detect DNA or RNA coding for 1-173 splice variants of PTHRP. Here again, once it is recognized that such variants should be investigated, assays for performing such detection can be performed using standard procedures, and fall well within the ordinary skill of the art.

The invention also provides for *in vivo* diagnostic assays wherein one or more antibodies or other eptitope-specific binding molecules of the invention are administered to a subject. Preferably, antibodies are labeled with a detectable marker to provide for the detection of cells to which the labeled antibodies of the invention have bound, examples of such labels include radionuclides and radio-opaque imaging agents. Such methods of diagnosis may comprise the step of administering detectably labeled antibodies to a subject, whereby complexes between the antibody and PTHRP form, and the step of detecting such complexes.

The assays of the invention may be either *in vitro* or *in vivo*. The assays of the invention also include assays for the detection of PTHRP expressing cells by immunohistochemistry techniques such as those techniques described in Cuello Immunohistochemistry II, John Wiley & Sons (1993), Bullock, *et al.*, Techniques In Immunocytochemistry Volumes 1 and 2, Academic Press (1983), wherein the antibodies bound to PTHRP may be visualized by fluorescence or similar techniques. Immunohistochemistry techniques may comprise the step of incubating a tissue sample with a labeled antibody (labeled either directly or indirectly) of the invention and detecting where in the tissue sample the antibody binds by means of the label. In some embodiments of the assays of the invention the assays comprise the step of adding an antibody to a sample for analysis and measuring the binding of the antibody to the PTHRP in the sample for analysis. The antibodies used in such assays may be may be labeled directly or indirectly. In other embodiments of the assays of the invention, PTHRP in the sample for analysis interferes with the binding of the antibody of the invention to PTHRP of a target molecule of the invention. The antibodies of the invention may also be used in conjunction with flow cytometry so as to identify PTHRP producing or non-producing cells.

Another aspect of the invention provides kits for performing one or more of the various aspects of the invention. Such kits may contain one or more reagents necessary for performing the immunoassays of the invention,

and may contain the subject compounds and reagents in pre-measured amounts so as to ensure both precision and accuracy when performing the subject methods. Kits may also contain instructions for performing the methods of the invention. The kits of the invention may include one or more of the following items: antibodies of the invention, target molecules of the invention, standards of known concentration, buffers, antibody labeling reagents, reaction vessels, and the like.

In a preferred embodiment, the kits of the invention comprise at least two reagents that may be used in performing the subject assays. Kits for performing the sandwich assay may comprise an antibody of the invention having the property of specifically binding PTHRP 109-141, and an antibody of the invention having the property of specifically binding PTHRP 140-173. The kits of the invention may further comprise a target molecule of the invention. The antibodies and target molecules included in the subject kits may be labeled with a detectable marker. The kits of the invention may also comprise various other reagents useful for carrying out the assays of the invention, such reagents include buffers, solid supports, enzymatic substrates, secondary labeling antibodies, positive controls for PTHRP, negative controls for PTHRP, and the like.

TREATMENT

Another aspect of the invention provides methods of treating neoplasms and other diseases associated with

production of PTHRP. The term "treatment" as used herein refers not only to cures, but also to any alleviation of symptoms or reduction in the rate of progression of disease.

Treatment methods may include the administration of an effective amount of one or more of the eptitope-specific binding molecules of the invention, including PTHRP specific antibody. Such eptitope-specific binding molecules may be labeled or unlabeled, and may be complexed with a therapeutic agent such as a toxin, a radionuclide, an anti-cancer drug, and the like. Antibodies of the invention for therapeutic use are preferably antibodies derived from the same species as the species of the animal to be treated. For example, when the subject to be treated is human, the PTHRP specific antibodies to be administered to the patient are preferably human antibodies or "humanized" antibodies, e.g. CDR-grafted antibodies as described in Winter US patent 5,225,539 or chimeric antibodies as described in European patent application EP120694 (CellTech). In addition to providing treatment methods in which neoplastic cells are killed or stopped from replicating, the invention also provides methods of treatment wherein antibodies or epitope-specific binding molecules are used to remove or "inactivate" PTHRP in the general circulation thereby reducing the hypercalcemia associated with PTHRP secretion.

Another aspect of the invention provides pharmaceutical formulations comprising the antibodies or other eptitope-specific binding molecules of the invention. The subject

pharmaceutical formulations may be used for both treatment and *in vivo* diagnosis, depending upon the precise formulation. The antibodies of the invention may be administered by a number of methods e.g., topically, orally, intranasally, by injection or by inhalation. The antibodies of the invention may also be used with one or more inert carrier materials.

As the preferred means for administering the antibodies of the invention is parenterally, the preferred formulations of the invention are optimized for parenteral administration. For parenteral administration by injection, the formulations may comprise an aqueous solution of a water soluble pharmaceutically acceptable salt of the active acids according to the invention, desirably in a concentration of 0.05 - 10%, and optionally also a stabilizing agent and/or buffer substances in aqueous solution. Dosage units of the solution may advantageously be enclosed in ampoules.

The dosage at which the antibodies of the invention are administered may vary within a wide range and will depend on various factors such as, for example, whether or not the antibody is being used for diagnosis or therapy, the severity of the disease, the age of the patient, the binding affinity of the particular antibody for PTHRP, etc., and may have to be individually adjusted. A possible starting range for the amount of antibody which may be administered per day is about 0.001 mg to about 200 mg.

In one method of treatment, excess PTHRP is removed from a patient by removing blood from the body, treating the blood

with antibodies to remove PTHRP, and reintroducing the treated blood into the body.

The following examples are offered for the purposes of illustrating the subject invention and are not offered by way of limitation.

**EXAMPLES**

A container or bead, preferably plastic, is treated with antibody, preferably monoclonal, to epitope amino acid 140-173 of parathyroid hormone-like protein causing the antibody to adsorb to the surface of the container or bead. A serum sample containing an unknown level of parathyroid-like hormone is added to the coated container or bead along with a labeled derivative, preferably monoclonal, to epitope amino acid 109-141 of parathyroid hormone-like protein. The mixture is incubated to allow complexation of the two antibodies and the parathyroid hormone-like protein, the solid phase is washed to remove uncomplexed material and the label detected. The signal is directly proportional to the parathyroid hormone-like protein level of the serum sample, and when compared to calibrators of known concentration, a specific determination of the parathyroid hormone-like protein level can be made.

In an alternative embodiment, a competitive assay may be used, as for example, one in which labeled parathyroid hormone-like protein or a peptide of the 140-173 epitope can be added to the above described solid phase and serum sample. The endogenous parathyroid hormone-like protein compete with

this labeled material to bind to the solid phase. After washing and label detection, the amount of parathyroid hormone-like protein in the sample, which is inversely proportional to the signal detected, can be determined using a calibration curve of known parathyroid hormone-like protein concentrations.

Both of the above example methods can be accomplished in the liquid phase with subsequent binding or precipitation to separate antibody bound label. The solid phase may or may not be paramagnetic.

In yet other embodiments, experiments were performed in which monoclonal antibodies raised against different portions of PTHRP were tested for their ability to detect PTHRP in neoplastic cells. The antibodies tested were prepared in response to the peptides consisting of amino acid residues 1-34 of PTHRP, 109-141 of PTHRP, and 140-173 of PTHRP. The monoclonal antibodies were obtained from the laboratory of Dr. Leonard Deftos, University of California, San Diego, Veterans Administration Hospital. The PTHRP 109-141 specific antibody was produced by hybridoma 9H7. The PTHRP 140-173 specific antibody was produced by hybridoma 1D5. These experiments showed that in both immunoassay and immunohistochemical procedures, the antibodies demonstrated the expression of their respective epitopes of PTHRP in a variety of neoplastic cells including surgical specimens and tissue cultures, and in particular in several different breast cancer cell lines. In the latter case, reverse transcriptase PCR (RT-PCR) was

performed in order to demonstrate that PTHRP encoding mRNA was produced in the cell line.

**EQUIVALENTS**

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the fields of infectious disease, biochemistry, or related fields are intended to be within the scope of the following claims.

**INCORPORATION BY REFERENCE**

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

## CLAIMS

What is claimed is:

1. An eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope on PTHRP 140-173.

2. The ESBM according to Claim 1 comprising an antibody.

3. The ESBM according to Claim 1 comprising a monoclonal antibody.

4. The ESBM according to Claim 3 wherein said monoclonal antibody is raised in response to a peptide consisting of at least a portion of amino acids 140-173 of PTHRP.

5. An ESBM according to Claim 1, wherein said monoclonal antibody is produced by a recombinant host cell.

6. The ESBM according to Claim 1 wherein said monoclonal antibody is produced by hybridoma 1D5.

7. An eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope on PTHRP 104-141.

8. The ESBM according to Claim 1 comprising an antibody.

9. The ESBM according to Claim 1 comprising a monoclonal antibody.

10. The ESBM according to Claim 3 wherein said monoclonal antibody is raised in response to a peptide consisting of at least a portion of amino acids 140-173 of PTHRP.

11. An ESBM according to Claim 1, wherein said monoclonal antibody is produced by a recombinant host cell.

12. The ESBM according to Claim 1 wherein said monoclonal antibody is produced by hybridoma 9H7.

13. A method of detecting PTHRP in a sample comprising:
providing an eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope of PTHRP 140-173;
adding said ESBM to said sample to form a complex; and
measuring the amount of said complex formation.

14. A method of detecting PTHRP in a sample comprising:
providing an eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope of PTHRP 104-141;
adding said ESBM to said sample to form a complex; and
measuring the amount of said complex formation.

15. A method of detecting PTHRP in a sample comprising: providing a first ESBM which specifically binds to at least a first epitope of PTHRP 140-173;

providing a second ESBM which specifically binds to at least a second epitope of PTHRP 104-141;

adding said first and second ESBMs to said sample to form a complex; and

measuring the amount of said complex formation.

16. The methods of any of claims 13-15 further comprising providing at least one of the ESBMs as a monoclonal antibody.

17. The methods of any of claims 13-15 wherein said sample is a tissue sample and further comprising identifying the binding site of the antibodies by means of the label.

18. The methods of any of claims 13-15 further comprising labeling at least one of the ESBMs with a detectable marker.

19. The methods of any of claims 13-15 further comprising labeling at least one of the ESBMs with a detectable marker comprising at least one of an enzyme, biotin, a hapten, a fluorophore, a chromophore, a heavy metal, a paramagnetic isotope, colloidal gold, a radioisotope, and a lumiphore.

20. The methods of any of claims 13-15 wherein said sample is a tissue sample.

21. A polypeptide comprising the amino-acid sequence PTHRP 140-173.

22. A polypeptide comprising the amino-acid sequence PTHRP 109-141.

23. A target molecule capable of specifically binding to one of the ESBMs of claims 1-12.

24. The target molecule of claim 21 wherein the target molecule is a polypeptide.

25. The target molecule of claim 22 further comprising a subfragment of one of PTHRP 140-173 and PTHRP 109-141.

26. The target molecule of claim 21 further comprising a derivative of one of PTHRP 140-173 and PTHRP 109-141.

27. An assay comprising:

an eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope of PTHRP 140-173.

28. An assay comprising:

an eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope of PTHRP 104-141.

29. A sandwich assay comprising:

a first eptitope-specific binding molecule (ESBM) which specifically binds to at least one epitope of PTHRP 140-173; and

a second (ESBM) which specifically binds to at least one epitope of PTHRP 104-141.

30. An assay for detecting a 1-173 splice variant of PTHRP in a sample comprising:

providing an ESBM which specifically binds to the splice variant;

adding said ESBM to said sample to form a complex; and

measuring the amount of said complex formation.

31. An assay for detecting a 1-173 cDNA encoding for PTHRP in a sample comprising:

providing an ESBM which specifically binds to the splice variant;

adding said ESBM to said sample to form a complex; and

measuring the amount of said complex formation.

32. An assay for detecting a 1-173 RNA encoding for PTHRP in a sample comprising:

providing an ESBM which specifically binds to the splice variant;

adding said ESBM to said sample to form a complex; and

measuring the amount of said complex formation.

33. The assay of any of claims 27-32 wherein at least one of the ESBMs is a monoclonal antibody.

34. The assay of any of claims 27-32 wherein at least one of the ESBMs is labeled with a detectable marker comprising at least one of an enzyme, biotin, a hapten, a fluorophore, a chromophore, a heavy metal, a paramagnetic isotope, colloidal gold, a radioisotope, and a lumiphore.

35. A kit according to any of claims 27-32, said kit further comprising reagents in pre-measured amounts, instructions and at least one reaction vessels.

36. A method of treating a disease characterized by the production of parathyroid hormone-related protein, said method comprising:

administering an effective amount of one or more of the ESBMs of claims 1-12 to a patient.

37. The method of claim 36 further comprises conjugating at

least one of the ESBMs with at least one of a toxin, a radionuclide, and an anti-cancer drug.

38. The method of claim 36 comprises providing at least one of the ESBMs as human antibodies and humanized antibodies.

39. The method of claim 36 further comprising using at least one of the ESBMs to remove or "inactivate" PTHRP in the general circulation of the patient.

# INTERNATIONAL SEARCH REPORT

Inte...tional application No.
PCT/US96/10090

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :G01N 33/53; C07K 14/00, 9/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | Chemical Abstracts, Volume 118, Number 15, issued 12 April 1993, Kasahara et al., "Immunoassay of parathyroid hormone-related protein (PTHrP) in body fluid for diagnosis," page 180, column 1, abstract No. 118: 140690e, see entire document. | 14, 18, 19, 28, 34<br>---<br>15-17, 20, 33, 35 |
| X<br>---<br>Y | THE NEW ENGLAND JOURNAL OF MEDICINE, Volume 322, Number 16, issued 19 April 1990, Burtis et al., "Immunochemical Characterization of Circulating Parathyroid Hormone-Related Protein in Patients with Humoral Hypercalcemia of Cancer", pages 1106-1112, see pages 1108-1109, Figures 1 and 3. | 14, 18, 19, 28, 34<br>---<br>15-17, 20, 33, 35 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>05 SEPTEMBER 1996 | Date of mailing of the international search report<br>02 OCT 1996 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>CAROL A. SPIEGEL<br>Telephone No. (703)308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10090

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | BIOCHEMISTRY, Volume 33, Number 23, issued 14 June 1994, Yang et al., "Parathyroid Hormone-Related Protein: Evidence for Isoform- and Tissue-Specific Posttranslational Processing", pages 7460-7469, see abstract; pages 7461, 7463, 7467-7468. | 14, 18, 19, 28, 34<br>---<br>1-13, 15-17, 20, 27, 29, 33, 35 |
| X<br>---<br>Y | CANCER RESEARCH, Volume 53, Number 8, issued 15 April 1993, Iwamura et al., "Immunohistochemical Localization of Parathyroid Hormone-related Protein in Human Prostate Cancer", pages 1724-1726, see entire document. | 14, 16-20, 28, 33, 34<br>---<br>1-13, 15, 27, 29, 35 |
| Y | CLINICAL CHEMISTRY, Volume 38, Number 11, issued 1992, Burtis, "Parathyroid Hormone-Related Protein: Structure, Function, and Measurement", pages 2171-2183, see pages 2173-2174, 2179-2180. | 1-20, 27-29, 33-35 |
| Y | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 85, issued January 1988, Mangin et al., "Identification of a cDNA encoding a parathyroid hormone-like peptide from a human tumor associated with humoral hypercalcemia of malignancy", pages 597-601, see abstract; Figure 2. | 1-20, 27-29, 33-35 |

# INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US96/10090

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 23, 36-39
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, 27-29 and 33-35

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10090

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.5, 7.9, 7.94, 40.52, 69.4; 436/63, 64, 548; 530/388.24, 389.2

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/7.1, 7.5, 7.9, 7.94, 40.52, 69.4, 973, 975; 436/63, 64, 548, 808; 530/388.24, 389.2; 930/DIG 820

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, JPO, DIALOG
search terms: parathyroid hormone related/like protein, PTHRP, antibody, C-terminal

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.
Group I, claim(s)1-20, 27-29 and 33-35, drawn to two products which specifically bind to parathyroid hormone-related protein (PTHRP) epitopes, one to epitope 140-173 and the other to epitope 104-141, and their use, alone or together, in a one- or two-site immunoassay for detecting PTHRP.
Group II, claim(s) 1-12, 30 and 33-35, drawn to two products which specifically bind to parathyroid hormone-related protein (PTHRP) epitopes, one to epitope 140-173 and the other to epitope 104-141, and their use in detecting a splice variant of PTHRP.
Group III, claim(s) 1-12, 31 and 33-35, drawn to two products which specifically bind to parathyroid hormone-related protein (PTHRP) epitopes, one to epitope 140-173 and the other to epitope 104-141, and their use in detecting a cDNA encoding PTHRP.
Group IV, claim(s)1-12, 32 and 33-35, drawn to two products which specifically bind to parathyroid hormone-related protein (PTHRP) epitopes, one to epitope 140-173 and the other to epitope 104-141, and their use in detecting an RNA encoding PTHRP.
Group V, claim(s) 1-12, drawn to two products which specifically bind to parathyroid hormone-related protein (PTHRP) epitopes, one to epitope 140-173 and the other to epitope 104-141, and their use in treating disease.Group VI, claim(s) 21, drawn to a thirdproduct, PTHRP 140-173 polypeptide.Group VII, claim(s)22, drawn to a fourth product, PTHRP 109-141 polypeptide.
Group VIII, claim(s) 24-26, 1-6 and 8-11, drawn to a first species of target molecule capable of specifically binding to a first product which specifically binds to PTHRP epitope 140-173.
Group IX, claim(s) 24-26, 7 and 12, drawn to a second species of target molecule capable of specifically binding to a second product which specifically binds to PTHRP epitope 104-141.
The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The last paragraph on page 4 of the disclosure characterizes the special technical features of the invention as "[exploiting] the surprising discovery that monoclonal antibodies raised in response to a peptide consisting of amino acid residues 140-173 of PTHRP and monoclonal antibodies raised in response to a peptide consisting of amino acid residues 109-141 of PTHRP, either alone or in combination, are able to detect PTHRP expressing neoplastic cells with a much higher sensitivity than other monoclonal antibodies that are capable of binding to other regions of the PTHRP molecule, and the detection of PTHRP with such monoclonal antibodies was better correlated with the presence of neoplasia, especially breast cancer and prostate cancer, than other PTHRP specific antibodies". The products of Groups VI-IX are separate and distinct products, i.e.polypeptides and target molecules, which do not share this special technical feature, i.e. monoclonal antibodies raisedinresponse to a peptide consisting of amino acid residues 140-173 of PTHRP and monoclonal antibodies raised in response to a peptide consisting of amino acid residues 109-141 of PTHRP.
Furthermore, PCT Rule 13 permits, in particular, (i) in addition to an independent claim for a given product, an independent claim for a use of said product. Groups I-V each recite a separate and distinct use for the special technical feature of the invention.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10090

Claims 23 and 36-39 have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.